Pulmonary Function

Results of the respiratory function measurements are presented in Tables F9 through F41. A progressive dose and time-related impairment of respiratory function was observed in both male and female rats exposed to talc. The impairment was restrictive in nature, consisting of reduced lung volume, increased lung stiffness, reduced gas exchange efficiency, and nonuniform intrapulmonary gas distribution.

6-Month Interim Evaluation: At 6 months there were few significant differences between values for rats exposed to 18 mg/m^3 and controls, and no significant differences between values for rats exposed to 6 mg/m^3 and controls. There were, however, slight trends among both males and females toward smaller lung volumes and reduced forced expiratory flow. Total lung capacity, vital capacity, and forced vital capacity were all slightly smaller in the 18 mg/m^3 groups, but only the forced vital capacity of females differed significantly from controls. All forced expiratory flow rates were lower in the 18 mg/m^3 groups, but only those of males were significantly lower than those of the controls. The reduced flow rates were partly related to the smaller lungs, but even volume-normalized flow tended to be reduced in the exposed rats. The reduced flow rates most likely reflected changes in small airways. Total pulmonary resistance, which primarily reflects flow resistance in large airways, was unaffected.

11-Month Interim Evaluation: Functional alterations were clearly apparent in exposed males and females after 11 months. Total lung capacity, vital capacity, and forced vital capacity were significantly lower in males and females exposed to 18 mg/m^3 and males exposed to 6 mg/m^3. The reduced volume was accompanied by significant reductions in quasistatic lung compliance in males, and both dynamic and quasistatic lung compliance in females. The volume and compliance changes indicate a stiffening of the lung (or increase in elastic recoil). Forced expiratory flows during mid to late expiration were slightly lower in exposed males than in controls, but the differences were not significant.

A reduction of alveolar-capillary gas exchange efficiency was reflected by a significant reduction of carbon monoxide diffusing capacity in the 18 mg/m^3 male and female rat groups. Although diffusing capacity is somewhat volume dependent, the reduced lung volume did not completely account for the change. Volume-normalized diffusing capacity was also significantly reduced in male and female rats exposed to 18 mg/m^3.

18-Month Interim Evaluation: Total lung capacity, vital capacity, and forced vital capacity of all exposed groups of male and female rats were significantly lower than those of controls at 18 months, except for vital capacity of males exposed to 6 mg/m^3. In females exposed to 18 mg/m^3, these decreases were accompanied by significant increases in resting (functional residual capacity) and minimum (residual) volumes. The decrease in volume at maximum inflations (total capacity, vital capacity, and forced vital capacity) reflected the inability of the stiffened lungs to stretch normally. Volume-normalized forced expiratory flows of exposed male and female rats were generally greater than those of controls, due to the reduced lung volume and little or no reduction in flow.

All parameters of lung compliance in male and female rats exposed to 18 mg/m^3 were also significantly lower than controls at 18 months, while two of the three compliance parameters were significantly lower at the 6 mg/m^3 exposure level. The carbon monoxide diffusing capacities in males and females exposed to 18 mg/m^3 were significantly lower than controls at 18 months, which is consistent with the findings at 11 months.

The slope of phase III of the single-breath N_2 washout of male and female rats exposed to 18 mg/m^3 was significantly greater than controls, apparently due to uneven mixing of oxygen with residual nitrogen in the lung during maximal inflation. This finding reflects a nonuniform distribution of inhaled air.

24-Month Interim Evaluation: Because of reduced survival in all groups of male and female rats, fewer animals remained alive at 24 months for evaluation of pulmonary function. Because of the smaller group sizes (3 rats each from the control and 18 mg/m^3 groups were evaluated), few of the differences were statistically significant. Nevertheless, there were reductions in lung volume parameters (total lung capacity, vital capacity, and forced vital capacity), lung compliance, and carbon monoxide diffusing capacity in exposed male and female rats consistent with the findings at the earlier time periods.

The progression of the functional impairments over the course of the study are illustrated in Figure 3, which plots the data for three functional parameters obtained from the 3 male and 3 female rats in the 18 mg/m^3 exposure groups surviving until 24 months.

Bronchoalveolar Lavage and Lung Biochemistry

Following the completion of the pulmonary function tests at the 24-month interim evaluation, bronchoalveolar lavage was performed on the remaining rats in these groups and the lavage fluid was evaluated for enzymes, protein, and cell content as shown in Tables F4 and F5. Values for glucose-6-phosphate dehydrogenase and glutathione peroxidase are not reported because they were below the limits of detection.

The values for ß-glucuronidase, alkaline phosphatase, lactate dehydrogenase, and total protein in both male and female rats exposed to 18 mg/m^3 talc were significantly greater than those of controls. In addition, females in this group had a significantly higher value for glutathione reductase. Both male and female rats exposed to 6 mg/m^3 talc had significantly greater ß-glucuronidase values, but only female rats exposed to 6 mg/m^3 had higher values of alkaline phosphatase, lactate dehydrogenase, and protein. The percentages of polymorphonuclear leukocytes in the lavage fluid were also significantly greater in male and female rats exposed to talc at both concentration levels. The increase in enzymes, total protein, and leukocytes are consistent with the morphological findings of a chronic active inflammatory process and cellular degenerative changes.

The viability and phagocytic activity of alveolar macrophages recovered from the lungs of rats exposed to 6 or 18 mg/m^3 talc or from the chamber controls ranged from approximately 60% to 80%. Neither the viability or phagocytic activity were significantly affected by exposure to talc (Table F6).

Table F7 summarizes the effects of talc exposure on collagen metabolism and protein synthesis. Collagenous peptides in lavage fluid and collagen production (% newly synthesized protein) from female rats, but not males, exposed to 6 or 18 mg/m^3 were significantly greater than controls. Total lung collagen from males and females at both exposure levels were also significantly greater. Values for non-collagenous protein synthesis were significantly greater in males exposed to 6 or 18 mg/m^3 and in females exposed to 18 mg/m^3 than in controls.

Lung proteinase activity, as determined from lavage fluid and homogenate supernatant fluid, is shown in Table F8. Acid proteinase activity, primarily cathepsin D, was significantly greater in both males and females exposed to 6 or 18 mg/m^3 than in controls. Neutral proteinase activity in homogenate supernatant fluid was also greater in rats exposed to talc. The activity was mostly serine proteinase, like that of polymorphonuclear leukocyte elastase and cathepsin G.

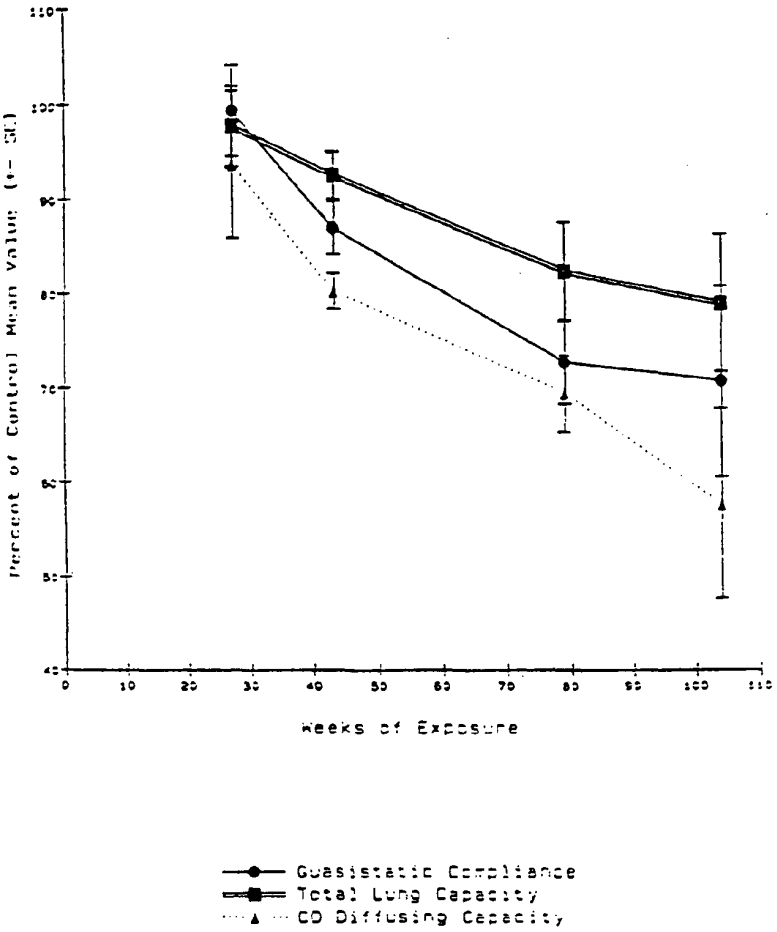


FIGURE 3
Effect of 18 mg/m³ Talc Exposure on Respiratory Function of Male and Female Rats Surviving to 104 Weeks

MICE

4-WEEK STUDY DOSE SELECTION

Selection of 6 or 18 mg talc/m^3 as the exposure concentrations was based on the results of a 4-week inhalation study in B6C3F$_1$ mice to determine lung talc burden and histopathologic changes associated with talc exposure. These studies indicated that the amount of talc retained in the lung was similar between sexes and proportional to exposure concentration (Appendix K). Microscopic examination of the lungs revealed an accumulation of alveolar macrophages in the lungs only at 18 mg/m^3. Based on these findings it was predicted that aerosol concentrations greater than 18 mg/m^3 would overwhelm lung clearance mechanisms, impair lung function, and possibly shorten survival.

2-YEAR STUDY

Survival

Estimates of survival probabilities for male and female mice are shown in Table 8 and in the Kaplan-Meier curves in Figure 4. Survival of male and female mice exposed to talc was similar to that of the controls throughout most of the study. One female mouse exposed to 18 mg/m^3 died on day 20 and six others died on day 28 of the study of undetermined cause.

Body Weights and Clinical Findings

Mean body weights of male and female mice exposed to talc were similar to controls throughout the study (Tables 9 and 10, and Figure 5). There were no clinical findings in exposed mice that could be attributed to exposure to talc.

Prior to the start of the study and after 6 months of exposure, serological tests were negative for all viruses tested and *Mycoplasma spp*. At 12 months, 8/24 mice were positive for mouse hepatitis virus (MHV), but retesting of the serum by the enzyme linked immunosorbent assay (ELISA) showed all to be negative. At the end of the study, 7/30 were positive for *Mycoplasma arthritidis* and 21/30 were positive for epizootic diarrhea of infant mice (EDIM). No clinical signs or gross or microscopic evidence of disease associated with *M. arthritidis* was observed. EDIM does not cause clinical disease or pathology in adult mice.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the lung, lymph node, and nose. Summaries of the incidences of nonneoplastic lesions and neoplasms, the individual animal neoplasm diagnoses, and the statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one group are presented in Appendix C for male mice and Appendix D for female mice.

TABLE 8
Survival of Mice in the 2-Year Inhalation Study of Talc

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Core Study Groups			
Animals initially in study	50	50	50
Natural deaths	16	18	14
Moribund kills	1	2	3
Missing[a]	2	1	1
Missexed[a]	1	1	0
Animals surviving to study termination	30	28	32
Percent survival at end of study[b]	65	58	66
Mean survival (days)[c]	648	648	645
Survival analysis[d]	P=0.886N	P=0.771	P=1.000N
Special Study Groups[a]			
Animals initially in study	39	40	40
Natural deaths	4	5	7
Moribund kills	0	1	1
Missing[a]	0	1	1
Scheduled sacrifice	35	33	31
Females			
Core Study Groups			
Animals initially in study	50	50	50
Natural deaths	17	21	21
Moribund kills	2	4	4
Missing[a]	1	1	0
Culled[a]	0	1	0
Animals surviving to study termination	30	23	25
Percent survival at end of study[b]	62	48	50
Mean survival (days)[c]	663	648	590
Survival analysis[d]	P=0.321	P=0.322	P=0.286
Special Study Groups[a]			
Animals initially in study	39	40	40
Natural deaths	7	5	10
Moribund kills	2	5	1
Scheduled sacrifice	30	30	29

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

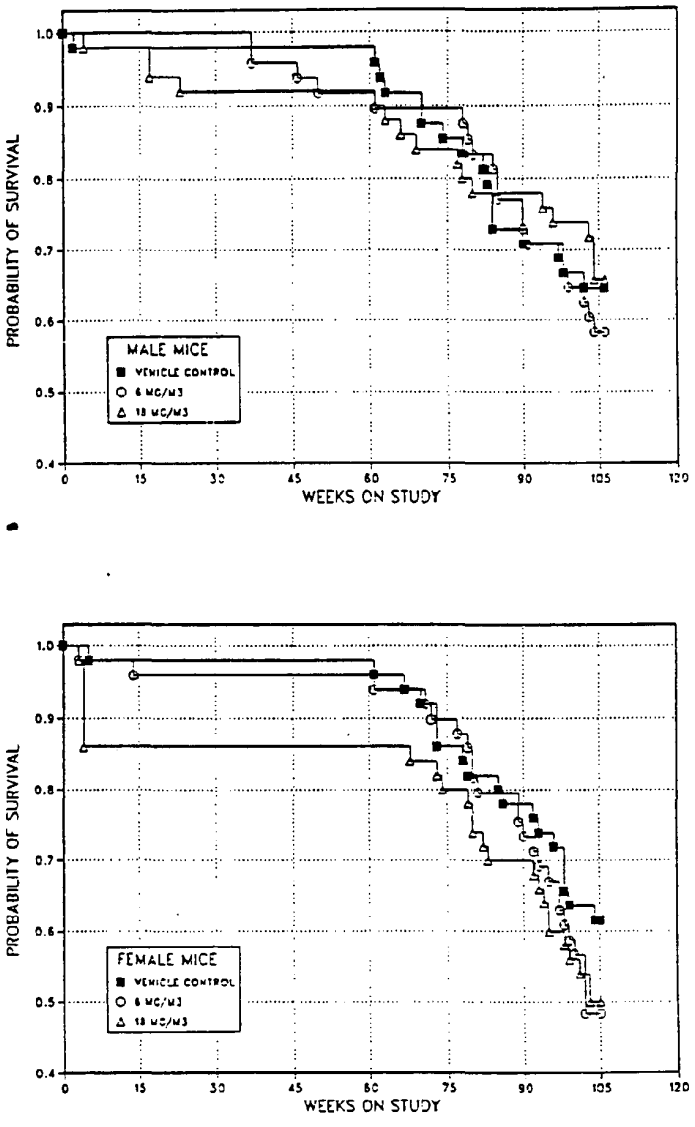


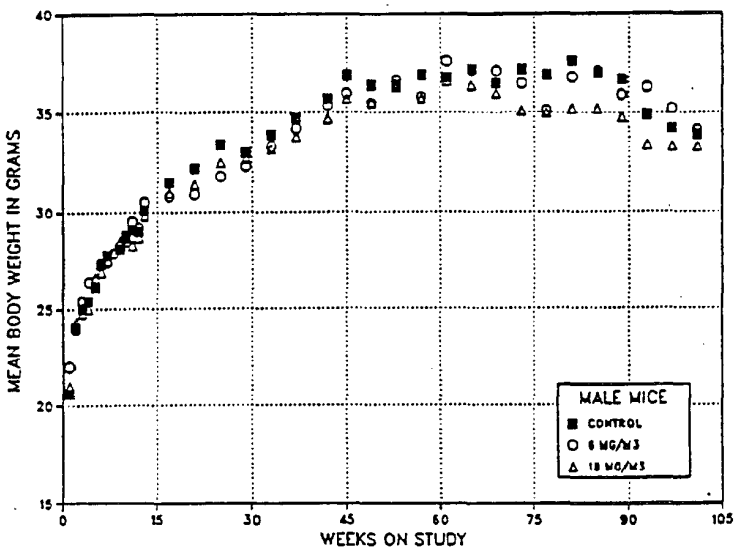
FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered Talc by Inhalation for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Talc

Week on Study	0 mg/m³ Av. Wt. (g)	0 mg/m³ Number of Survivors	6 mg/m³ Av. Wt. (g)	6 mg/m³ Wt. (% of controls)	6 mg/m³ Number of Survivors	18 mg/m³ Av. Wt. (g)	18 mg/m³ Wt. (% of controls)	18 mg/m³ Number of Survivors
1	23.3	50	23.8	102	50	23.7	102	50
2	24.0	48	23.9	100	49	24.3	101	50
3	25.0	47	25.4	102	49	24.8	99	50
4	25.4	47	26.4	104	49	25.0	98	50
5	26.1	47	26.2	100	49	26.6	102	49
6	27.3	47	27.4	100	49	26.9	99	49
7	27.8	47	27.4	99	49	27.5	99	49
8	25.8	47	27.9	108	49	29.7	115	49
9	28.1	47	28.3	101	48	28.5	101	49
10	28.8	47	28.5	99	48	28.7	100	49
11	29.1	47	29.5	101	48	28.3	97	49
12	29.0	47	29.2	101	48	28.7	99	49
13	30.1	47	30.5	101	48	29.8	99	49
17	31.5	47	30.8	98	48	31.0	98	47
21	32.2	47	30.9	96	48	31.4	98	47
25	33.4	47	31.8	95	48	32.5	97	46
29	33.0	47	32.3	98	48	32.7	99	46
33	33.9	47	33.3	98	48	33.2	98	46
37	34.7	47	34.2	99	46	33.8	97	46
42	35.7	47	35.4	99	46	34.7	97	46
45	36.9	47	36.0	98	46	35.7	97	46
49	36.4	47	35.5	98	45	35.5	98	46
53	36.4	47	36.6	101	44	36.3	100	46
57	36.9	47	35.8	97	44	35.7	97	46
61	36.8	46	37.6	102	43	36.6	100	45
65	37.2	44	37.1	100	43	36.4	98	44
69	36.5	44	37.1	102	43	36.0	99	42
73	37.2	42	36.5	98	43	35.1	94	42
77	36.9	41	35.1	95	43	35.0	95	42
81	37.6	40	36.8	98	40	35.2	94	39
85	37.0	35	37.1	100	37	35.2	95	39
89	36.7	35	35.9	98	37	34.8	95	38
93	34.9	34	36.3	104	34	33.4	96	38
97	34.2	33	35.2	103	34	33.3	97	36
101	33.9	31	34.1	101	31	33.3	98	36
Terminal sacrifice		30			28			32
Mean for weeks								
1-13	26.9		27.3	101		27.1	101	
14-52	34.2		33.4	98		33.4	98	
53-101	36.3		36.2	100		35.1	97	

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Talc

Week on Study	0 mg/m³ Av. Wt. (g)	0 mg/m³ Number of Survivors	6 mg/m³ Av. Wt. (g)	6 mg/m³ Wt. (% of controls)	6 mg/m³ Number of Survivors	18 mg/m³ Av. Wt. (g)	18 mg/m³ Wt. (% of controls)	18 mg/m³ Number of Survivors
1	19.3	50	19.3	100	50	19.6	102	50
2	19.9	50	20.1	101	50	20.5	103	50
3	21.0	50	21.3	101	50	21.1	101	50
4	22.4	50	22.5	100	49	21.5	96	49
5	22.5	49	22.7	101	49	23.2	103	43
6	24.4	49	23.7	97	49	23.8	98	43
7	24.6	49	24.5	100	49	24.3	99	43
8	22.1	49	24.2	110	49	26.8	121	43
9	24.6	49	24.9	101	49	25.2	102	43
10	25.2	49	25.4	101	49	25.3	100	43
11	25.6	49	26.2	102	49	25.0	98	43
12	25.5	49	25.1	98	49	25.2	99	43
13	26.3	49	26.4	100	49	25.9	99	43
17	27.5	49	26.7	97	47	27.3	99	43
21	28.4	49	27.2	96	47	27.7	98	43
25	29.5	49	28.1	95	47	28.9	98	43
29	29.8	49	28.6	96	47	28.9	97	43
33	30.1	49	29.7	99	47	29.5	98	43
37	30.7	49	29.9	97	47	29.9	97	43
42	31.7	49	30.8	97	47	30.3	96	43
45	32.4	49	31.7	98	47	31.1	96	43
49	32.2	49	31.2	97	47	31.0	96	43
53	32.7	49	31.4	96	47	31.9	98	43
57	32.7	49	31.0	95	47	31.2	95	43
61	33.1	49	32.9	99	46	32.3	98	43
65	33.0	48	32.4	98	46	32.7	99	43
69	32.7	47	32.4	99	46	32.1	98	42
73	32.8	43	32.1	98	44	31.0	95	41
77	32.6	43	31.3	96	43	31.3	96	40
81	33.5	41	32.7	98	39	32.1	96	37
85	32.5	40	33.0	102	39	32.7	101	35
89	32.7	39	32.1	98	36	32.1	98	35
93	31.7	37	31.7	100	33	31.2	98	33
97	31.5	35	31.7	101	30	30.6	97	30
101	31.8	31	31.4	99	27	31.0	98	27
Terminal sacrifice		30			23			25
Mean for weeks								
1-13	23.3		23.6	101		23.6	101	
14-52	30.3		29.3	97		29.4	97	
53-101	32.6		32.0	98		31.7	97	



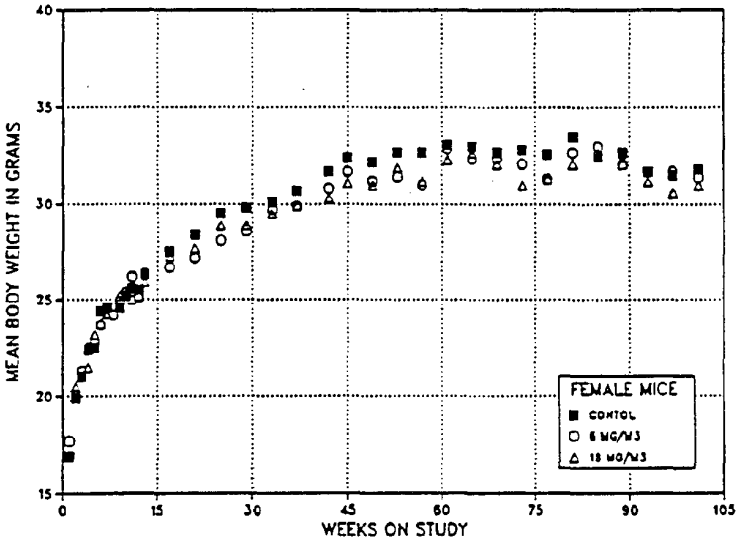


FIGURE 5
Growth Curves for Male and Female Mice Administered Talc by Inhalation For 2 Years

Lung: Absolute and relative lung weights of male and female mice exposed to 18 mg/m^3 talc were significantly greater at the 12- and 18-month interim evaluations and at the end of the study. Lung weights of mice exposed to 6 mg/m^3 were similar to controls at each of the interim evaluations.

The pulmonary lesions in mice exposed to talc were similar at the interim evaluations and at the end of the study, but the lesions varied in extent and severity with exposure concentration and duration (Table 11). The principal lung lesion occurring in exposed mice was an accumulation of alveolar macrophages in the alveoli surrounding terminal bronchioles (hyperplasia, macrophage) (Plate 8). The macrophages had abundant, slightly foamy to granular, eosinophilic cytoplasm containing birefringent talc particles. Small numbers of neutrophils were sometimes observed in the affected areas, and the interstitium contained infiltrates of mononuclear inflammatory cells (inflammation, chronic active) (Plates 9 and 10). In contrast to the pulmonary lesions in rats, hyperplasia of type II pneumocytes or fibrosis were not prominent components of the lesions in mice. The incidences of pulmonary neoplasms were similar among exposed groups and controls.

Lymph node: The bronchial lymph nodes of mice exposed to talc contained accumulations of macrophages in the medullary sinuses (hyperplasia, histocyte - male: 0 mg/m^3, 1/32; 6 mg/m^3, 32/39; 18 mg/m^3, 42/44; female: 0/38, 25/37, 39/43; Tables C4 and D4). The macrophages had abundant, slightly foamy to granular, eosinophilic cytoplasm filled with birefringent particles of talc.

Nose: The incidences of focal cytoplasmic alteration were increased in groups of mice exposed to talc (male: 5/45, 23/46, 40/47; female: 29/46, 37/46, 40/50; Tables C4 and D4). Focal cytoplasmic alteration was characterized by the formation of large eosinophilic droplets in the cytoplasm of olfactory and respiratory epithelial cells and was similar to that observed in rats.

Lung Talc Burden

The lung talc burdens, normalized to control lung weight or exposure level, are presented in Tables G2 and G3. Lung talc burden normalized to control lung weights (mg talc/g control lung) adjusts for differences in lung weight between sexes or at different ages. The lung burden normalized to control lung weight and exposure level (mg talc/g control lung/mg/m^3) adjusts for exposure level to determine the effect of exposure concentration on talc clearance from the lung.

The data, normalized to control lung weight, show that talc burdens of mice exposed to 6 mg/m^3 were similar between males and females and increased progressively from 6 to 24 months, except for males at 18 months (Table G2). However, because of the small sample size of males at 18 months (two animals), the lung talc burden of this sample may not be representative of the group as a whole. The lung talc burdens of mice exposed to 18 mg/m^3 were also similar between sexes at each interim evaluation. Although the talc burdens of males and females increased substantially from 6 to 24 months, the values at 12 and 18 months were similar.

The exposure-normalized data show that lung talc burdens of mice exposed to 18 mg/m^3 were disproportionately greater than those of mice exposed to 6 mg/m^3 (Table G2). The slight increases in exposure-normalized lung talc burden were statistically significant in males and females at 12 and 24 months, but not at 6 or 18 months. The lack of statistical significance at 18 months might be explained, in part, by the small sample size. These data suggest that clearance of talc from the lung was impaired, or impaired to a greater extent, in mice exposed to 18 mg/m^3 than in mice exposed to 6 mg/m^3.

Bronchoalveolar Lavage and Lung Biochemistry

Bronchoalveolar lavage was performed and lung homogenate supernatants collected for analyses at 6, 12, 18, and 24 months. A summary of the changes occurring in bronchoalveolar fluid enzymes, protein and cells are shown in Tables G4 through G22. Values for glucose-6-phosphate dehydrogenase, glutathione peroxidase, and alkaline phosphatase were not reported because they were below the limit of detection.

ß-Glucuronidase activity of lavage fluid from male and female mice exposed to 18 mg/m^3 was greater than that of controls at 12, 18, and 24 months, but not at 6 months. In mice exposed to 6 mg/m^3, ß-glucuronidase activity was greater than that of controls only at the 24-month interim evaluation. Lactate dehydrogenase and glutathione reductase activities in male and female mice exposed to

TABLE 11
Incidences of Nonneoplastic Lesions and Neoplasms in the Lung of Mice in the 2-Year Inhalation Study of Talc

	Male			Female		
	0 mg/m³	6 mg/m³	18 mg/m³	0 mg/m³	6 mg/m³	18 mg/m³
6-Month Interim Evaluation						
Lung[a]	4	4	4	4	4	4
Hyperplasia, Macrophage[b]	0	3 (1.0)[c]	4*(1.0)	0	0	4*(1.0)
Inflammation, Chronic Active	0	0	1 (1.0)	0	0	0
12-Month Interim Evaluation						
Lung	4	4	4	3	4	4
Hyperplasia, Macrophage	0	4*(1.0)	4*(1.8)	0	4*(1.0)	4*(2.0)
Inflammation, Chronic Active	0	0	2 (2.0)	0	0	1 (3.0)
18-Month Interim Evaluation						
Lung	4	4	4	4	4	4
Hyperplasia, Macrophage	0	4*(1.3)	4*(2.5)	0	4*(1.3)	4*(2.5)
Inflammation, Chronic Active	0	0	2 (1.5)	0	0	0
Alveolar/bronchiolar Adenoma	0	1	0	1	0	0
Alveolar/bronchiolar Carcinoma	1	0	0	0	0	0
2-Year Study						
Lung	45	47	48	46	48	50
Hyperplasia, Macrophage	3 (2.3)	46**(1.4)	48**(2.8)	2 (2.5)	45**(1.6)	43**(2.8)
Inflammation, Chronic Active	0	16**(1.1)	40**(2.2)	0	25**(1.4)	38**(2.3)
Alveolar Epithelium, Hyperplasia	1 (1.6)	0	0	0	0	1 (1.0)
Alveolar/bronchiolar Adenoma						
Overall rate[d]	6/45 (13%)	4/47 (9%)	9/48 (19%)	3/46 (7%)	2/49 (4%)	2/50 (4%)
Logistic regression[e]	P=0.251	P=0.411N	P=0.371	P=0.467N	P=0.499N	P=0.515N
Alveolar/bronchiolar Carcinoma						
Overall rate	7/45 (16%)	2/47 (4%)	2/48 (4%)	2/46 (4%)	4/49 (8%)	1/50 (2%)
Logistic regression	P=0.069N	P=0.073N	P=0.070N	P=0.325N	P=0.356	P=0.500N
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	12/45 (27%)	5/47 (11%)	11/48 (23%)	5/46 (11%)	6/49 (12%)	3/50 (6%)
Logistic regression	P=0.522N	P=0.043N	P=0.423N	P=0.269N	P=0.519	P=0.367N

* Significantly different (P≤0.05) from the control by Fisher's exact test (interim evaluation) or logistic regression (2-year study)
** P≤0.01
[a] Number of animals with lung examined microscopically.
[b] Number of animals with lesion.
[c] Average severity grades of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Number of animals with neoplasm per number of mice examined microscopically.
[e] Beneath the controls incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the control and that dosed group. The logistic regression tests regard these lesions as nonfatal. A negative trend or a lower incidence in a dosed group is indicated by N.

18 mg/m³ were significantly greater than those of controls at 18 and 24 months. Glutathione activity of males exposed to 18 mg/m³ was also greater than controls at 12 months. Values for total protein in lavage fluid from males and females in the 18 mg/m³ groups were significantly greater than controls at 18 months; at 24 months only that of males was significantly greater.

Significant differences in total and differential cell counts between exposed and control mice were observed only at 18 and 24 months at the high concentration level (Tables G8 to G11). The numbers of total nucleated cells, polymorphonuclear leukocytes, and macrophages were significantly greater in males and females exposed to 18 mg/m³ than in controls. Exposure of mice to 6 or 18 mg/m³ talc produced a concentration-related decrease in phagocytic activity of macrophages derived from lavage fluid (Tables G12 to G14). The number of macrophages containing phagocytized sheep erythrocytes from male and female mice exposed to 18 mg/m³ was significantly lower than that from control mice at 12, 18, and 24 months. Although phagocytic activity of macrophages from mice exposed to 6 mg/m³ was intermediate between controls and the high concentration groups, only the difference between the exposed and control males at 12 months was statistically significant.

The effects of talc exposure on lavage fluid collagenous peptides and total lung collagen are shown in Tables G15 through G18. The amount of collagenous peptides in lavage fluid from male mice exposed to 18 mg/m³ was significantly greater than that of controls at 12, 18, and 24 months, while collagenous peptides of females exposed to 18 mg/m³ were significantly increased only at 24 months. Consistent with these findings, total lung collagen was significantly greater in male mice at the high exposure concentration at 18 and 24 months and in females at 24 months. Collagenous peptides and total lung collagen from mice exposed to 6 mg/m³ were similar to controls at each of the interim evaluations.

The acid and neutral proteinase activity of lung homogenate supernatant fluid and the acid proteinase activity of lavage fluid are shown in Tables G19 through G22. Although there were no consistent exposure-related changes in lavage fluid acid proteinase activity at any of the interim evaluations, acid proteinase activity in supernatant fluid from male and female mice exposed to 18 mg/m³ was significantly greater than controls at 12, 18, and 24 months. The increase in acid proteinase activity was primarily due to cathepsin D-like activity. There were no consistent exposure-related changes in neutral proteinase activity at any of the interim evaluations.

DISCUSSION AND CONCLUSIONS

Talc ore may contain several other minerals including calcite, dolomite, magnesite, tremolite, anthophyllite, antigorite, quartz, pyrophyllite, micas, or chlorites. Since talc products are sold in a multitude of grades which have physical or functional characteristics especially suited for particular applications, occupational and consumer exposures to talc are complex. Exposure to industrial grade talc is known to cause pulmonary fibrosis, but the limited data on exposure to cosmetic grade talc are conflicting. Recently, epidemiology studies have revealed an association between nonfibrous talc and lung cancer risk (Thomas and Stewart, 1987). Talc was nominated by NIOSH for study by the NTP because of widespread human exposure and because of the lack of adequate information on its chronic toxicity and potential carcinogenicity.

The NTP toxicology and carcinogenicity studies of non-asbestiform, cosmetic grade talc, a finely powdered hydrous magnesium silicate, were conducted by exposing groups of male and female F344/N rats and $B6C3F_1$ mice to target aerosol concentrations of 0, 6 or 18 mg/m^3 talc for 6 hours daily, 5 days per week. Rats were exposed to talc until mortality in any group reached 80% (113 weeks for males and 122 weeks for females). Mice were exposed for 103 or 104 weeks. Exposure concentrations for the long-term studies were based on talc deposition and clearance patterns obtained from 4-week inhalation studies (Hanson *et al.*, 1985). In these studies, the amount of talc retained per unit of lung tissue was 79, 190, or 840 μg/g for male rats and 76, 185, or 770 μg/g for female rats exposed to 2, 6, or 18 mg/m^3. The amount of talc retained per unit of lung tissue in mice exposed at the same concentration levels were 130, 330, or 1,140 μg/g for males and 110, 330, or 1,160 μg/g for females. Only rats and mice at the highest exposure level had talc-containing macrophages within the alveolar spaces. Because there was a direct relationship between chamber concentration and lung talc burden and because of histologic evidence of a talc accumulation in alveolar macrophages at the 18 mg/m^3 concentration, it was predicted that higher levels would overwhelm lung clearance mechanisms in both species and cause deterioration of lung functions. Thus, 18 mg/m^3 was chosen as the top exposure concentration for the long-term studies.

The overall mean chamber concentrations achieved in the NTP long-term studies were 6.1 and 18.6 mg/m^3 for the rat study and 5.9 and 16.7 mg/m^3 for the mouse study. The average mass mean aerodynamic diameter of the talc particles was calculated to be 2.7 μm and 3.2 μm for the 6 and 18 mg/m^3 rat chambers and 3.3 μm and 3.6 μm for the 6 and 18 mg/m^3 mouse chambers, respectively. Seventy-five percent of the talc particles counted in four samples were in the 1 to 3 μm range. It has been shown, using aerosols of monodisperse aluminosilicate particles, in rats that particles larger than 10 μm are nearly all removed by inertial impaction in the nasal chamber or at bifurcation of the airways, while the percentage of particles deposited in the alveolar ducts and alveoli rises from almost zero at 10 μm to about 10% at about 1 μm (Raabe *et al.*, 1977) Thus, the large proportion of talc particles in these NTP studies were in the respirable range.

Because of difficulties with the aerosol concentration monitoring system for the 18 mg/m^3 rat chamber, there was a 7-week period beginning at study week 11 during which the chamber concentration for the high-dose rats varied from approximately 30 to 40 mg/m^3. Further, there was a 12-week period beginning at approximately week 70 during which there were difficulties in generating the talc aerosol and the chamber concentrations for rats and mice were substantially lower than the target concentrations (Figures H5 to H8). Although the exposure concentrations varied substantially from target concentrations during these periods, this does not preclude drawing conclusions regarding the chronic toxicity and carcinogenicity of talc. Since talc is a relatively inert particle, the amount of talc deposited and retained at the target site (lung talc burden) is a more relevant measure of talc exposure than chamber concentration. The problems with maintaining the target concentrations in the NTP studies did not have any apparent substantive effect on lung talc burdens.

The lung talc burden represents the difference between the amount of talc deposited in the lung and the amount removed by the clearance mechanisms. Inhaled particles deposited on the mucosal surface of the trachea, bronchi, or bronchioles are transported up the airways and from the lung through the ciliary activity of the respiratory epithelium, while particles reaching the alveolar region are phagocytized by alveolar macrophages and, to a lesser extent, other phagocytic inflammatory cells. Some of the alveolar macrophages migrate to the ciliated epithelium of the airways while others cross the alveolar lining to enter the interstitium and finally the lymphatics. Phagocytic cells reaching the lymphatics are transported in the lymph to the bronchial and mediastinal lymph nodes. Depending on the physiochemical properties of the inhaled particles, they may be partially or completely broken down within phagolysosomes of the macrophages and soluble components released from the cell. Talc is insoluble in water, cold acids, and alkalies and is likely to be insoluble in biological fluids. Talc particles were observed within macrophages in the lung and bronchial and mediastinal lymph nodes of rats and mice in these inhalation studies.

The lung talc burden of rats was greater than that of mice at each of the exposure concentrations and interim evaluations. The difference in lung talc burden is most likely related to anatomical and physiological differences known to influence particle deposition and retention including air flow pattern and velocity, respiratory rate, tidal volume, and clearance rate (McMahon *et al.*, 1977; Raabe *et al.*, 1977). The lung talc burdens of exposed rats and mice were generally similar between males and females at each exposure concentration and increased progressively with exposure duration. This indicated that the amount of talc deposited in the lung exceeded the clearance from the lung. The lung talc burden of rats was also generally proportional to exposure concentration at each interim evaluation, indicating that clearance of talc was not substantially impaired by increasing the exposure concentration, or that clearance of talc was impaired similarly at both exposure levels. In contrast, the lung talc burden of mice exposed to 18 mg/m^3 was disproportionately greater than that of mice exposed to 6 mg/m^3, indicating that clearance of talc from the lung was impaired, or impaired to a greater extent, in mice exposed to the higher concentration.

Analysis of bronchoalveolar lavage fluid has been used in human medicine for diagnosing the type or stage of various forms of interstitial lung disease and more recently as a rapid *in vivo* method of evaluating lung injury in toxicologic studies (Henderson *et al.*, 1985). Bronchoalveolar lavage was performed on rats and mice exposed to talc to evaluate its usefulness in chronic toxicology studies. Qualitatively similar changes in lavage fluid enzymes and cytology were observed in both species. Increases in neutrophils and total protein in lavage fluid are sensitive indicators of inflammation, and the increases in these parameters in rats and mice exposed to talc are consistent with the inflammation observed histologically in the lungs. Increases in cytoplasmic (lactate dehydrogenase and glutathione reductase) and lysosomal (ß-glucuronidase) enzymes, which are indicative of cellular injury, were also observed in both species. Whether lactate dehydrogenase and glutathione reductase were derived from parenchymal cells or inflammatory cells is unknown. The increase in glutathione reductase suggests that cellular injury may have involved an oxidative process involving free radicals produced during phagocytosis.

The phagocytic ability of alveolar macrophages recovered from lavage fluid was not impaired in rats exposed to talc for 24 months, as indicated by the lack of a significant difference in the number of viable macrophages and the percentage of cells phagocytizing sheep erythrocytes in exposed and control rats. In contrast, both the viability and the phagocytic ability of alveolar macrophages from exposed mice were significantly lower than those of macrophages from controls. The percent of macrophages containing phagocytized erythrocytes decreased as aerosol concentration and exposure duration increased. Since alveolar macrophages play a major role in the clearance of particles from the lung, the decreased viability and phagocytic ability of these cells may explain the disproportionately greater lung talc burden in mice exposed to 18 mg/m^3 than in mice exposed to 6 mg/m^3, and the difference in talc lung burdens between exposed rats and mice.

Due to limitations in chamber size and the number of animals that could be exposed, the numbers of animals utilized in the lung biochemistry studies were generally small. Therefore, some of the apparent inconsistencies in the results of these studies can be attributed to the small sample sizes as well as the biologic variation in pulmonary response among individuals. Despite these limitations, increases in lavage fluid collagenous peptides and total lung collagen were observed in both rats and mice exposed to 18 mg/m^3 talc. In rats, these

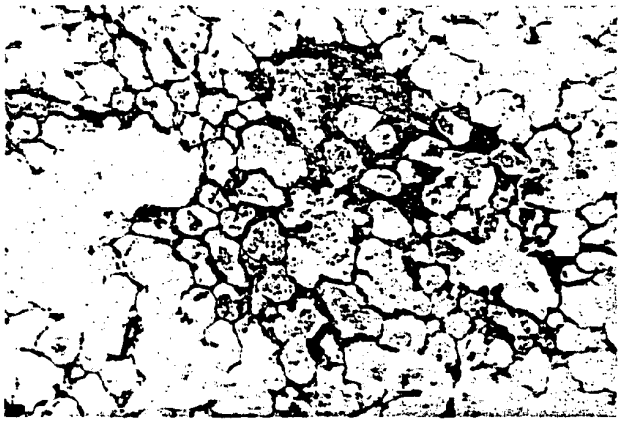


PLATE 1
Mild focal inflammation with thickening of the alveolar septa and distortion of the alveoli in lung of a male F344/N rat exposed to 18 mg/m^3 talc at the 18-month interim evaluation of the lifetime inhalation study. H&E, 25X

PLATE 2
Lung of a male F344/N rat exposed to 18 mg/m^3 talc at the 18-month interim evaluation of the lifetime inhalation study. Note the accumulation of alveolar macrophages with pale granular cytoplasm in the alveolar duct and slight thickening of the septal walls. H&E, 80X

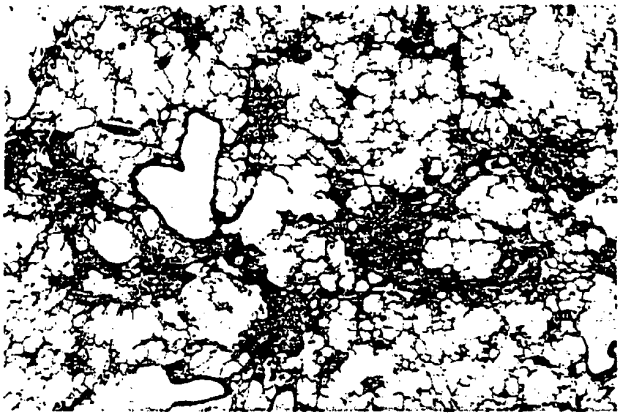


PLATE 3
Individual and confluent foci of interstitial fibrosis extend throughout the pulmonary parenchyma of a male F344/N rat exposed to 18 mg/m^3 talc at the 24-month interim evaluation of the lifetime inhalation study. H&E, 6.6X

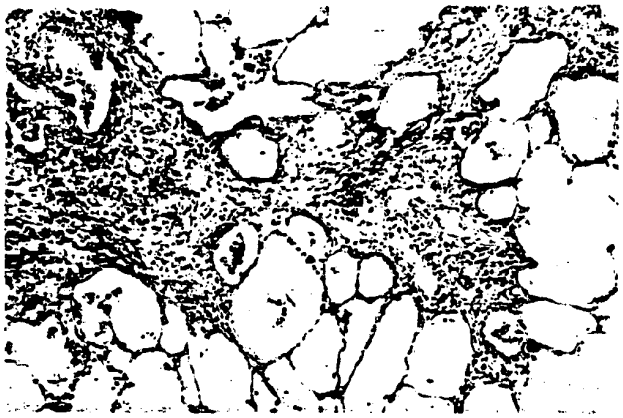


PLATE 4
Higher magnification of Plate 3 showing accumulation of fibrous tissue and interspersed inflammatory cells which obliterate the alveoli. H&E, 33X

PLATE 5
Squamous metaplasia and hyperplasia of the alveolar epithelium adjacent to an area of chronic inflammation and interstitial fibrosis in the lung of a male F344/N rat exposed to 18 mg/m^3 talc in the lifetime inhalation study. H&E, 40X

PLATE 6
Alveolar/bronchiolar carcinoma in a male F344/N rat exposed to 18 mg/m^3 talc in the lifetime inhalation study. Note the large mass obliterating the pulmonary parenchyma. H&E, 2.5X

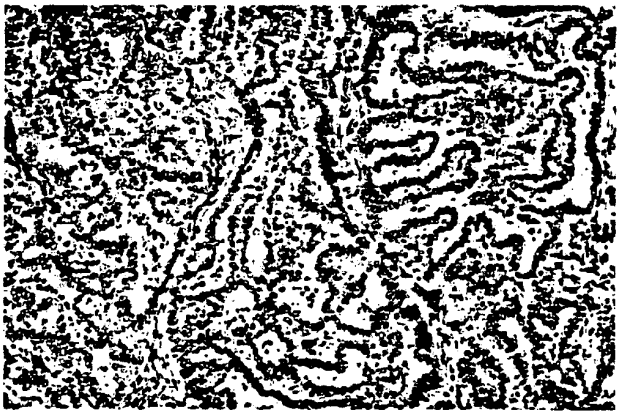


PLATE 7
Higher magnification of the alveolar/bronchiolar carcinoma in Plate 6 showing neoplastic epithelium arranged in irregular papillary formations. H&E, 50X

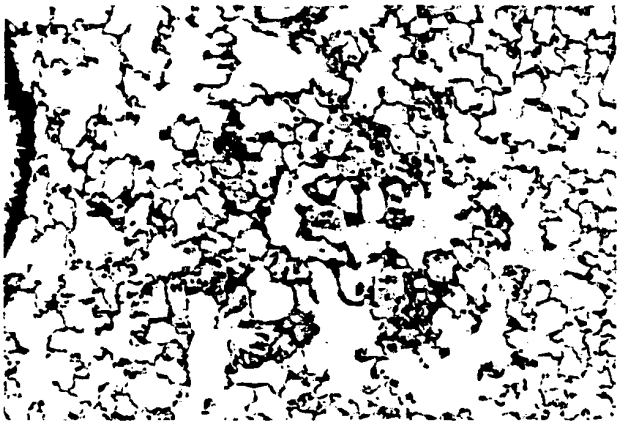


PLATE 8
Minimal focal accumulation of alveolar macrophages in the lung of a male B6C3F$_1$ mouse exposed to 18 mg/m^3 talc at the 12-month interim evaluation of the 2-year inhalation study. H&E, 50X

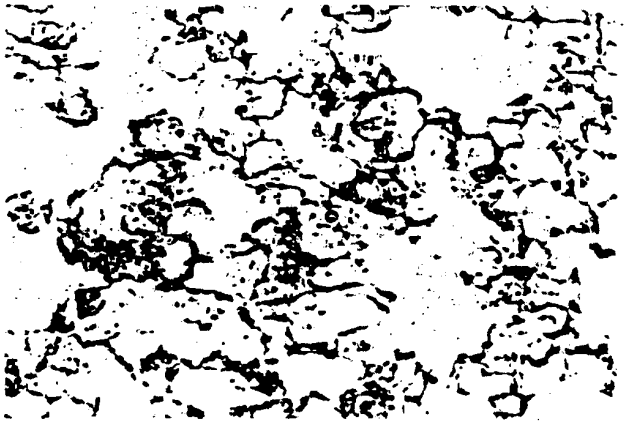


PLATE 9
Mile chronic active inflammation with slight thickening of the alveolar septa in the lung of a female B6C3F$_1$ exposed to 18 mg/m^3 talc in the 2-year inhalation study. H&E, 50X

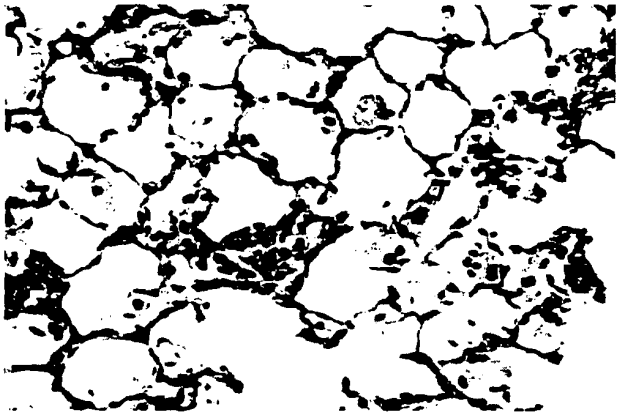


PLATE 10
Alveolar macrophages in alveoli and mononuclear cells in the interstitium of the lung of a male B6C3F$_1$ mouse exposed to 18 mg/m^3 talc in the 2-year inhalation study. H&E, 100X